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Consultants in Toxicology

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Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
P.O. Box 12233
Research Triangle Park, N.C. 27709.

Dear Dr. Jameson,

This pertains to the request in the Federal Register, March 19, 1998, for comments on the inclusion or delisting of substances in the Report on Carcinogens, Ninth Edition. I particularly refer to the questions raised concerning the delisting of saccharin.

It is now more than twenty years since the results of two-generation bioassays of sodium saccharin demonstrated that feeding high levels of this sweetener to the rat led to the development of bladder tumors. During these years, there has been a sustained scientific interest in saccharin. Many hundreds of studies have explored the biochemistry, pharmacokinetics, metabolism and toxicology of saccharin in man and animals and numerous epidemiological studies have been reported. A clear understanding of the research in all of these areas is essential in determining the risks that saccharin may pose to human safety, however, based on the transcript of the NTP Board Subcommittee meeting on saccharin, this grasp of the literature was missing. My comments are as follows:.

(1) Information that addresses the adequacy of existing epidemiological data, particularly as it relates to reported increased incidences of bladder tumor formation in certain small populations

There are now more than 30 human epidemiological studies in the literature that have examined relationships between saccharin consumption and bladder cancer. Reviews of these studies by Morgan and Wong (1985), IARC (1980, 1982), Armstrong (1985) and Elcock and Morgan (1993) have invariably concluded to the effect that "there is no consistent evidence that the risk of cancer is increased among users of saccharin". In the face of this vast body of literature attesting to the safety of saccharin in humans and the expertise represented in its assessments, it is surprising that questions concerning this aspect of saccharin safety studies would still be raised.

The press release of 10/31/97 mentioned "troubling subgroup findings in some of the epidemiological studies. It is not unusual that, among large series of epidemiological studies,

statistical analyses of subgroups will reveal some with statistically significant findings. Often, however, such results relate to relatively small numbers of subjects, are inconsistent within studies and between studies, and lack biological plausibility. Such is the case with the "troubling subgroup findings" in the artificial sweetener studies. I refer particularly to the Hoover and Strasser (1980) and Cartwright *et al.* (1981) case-control studies. In the former, relative risk of bladder cancer was significantly increased in heavy smoking males, whereas risk was not significantly increased in this subgroup in the Cartwright *et al.* study. Similarly, in the Cartwright *et al.* study, relative risk was not increased in non-smoking females, whereas a significantly increased relative risk was observed in this group in the Hoover and Strasser study. It is, perhaps, more important to recognise that Hoover and Strasser found that "in fact, for males and females, the lowest risk (of bladder cancer) was seen in the subjects with the longest use." These authors also concluded from their study that "inconsistencies in the data suggest that the positive associations may be due to chance."

Of all the case-control studies of saccharin, only one, Howe *et al.* (1977), reported an increased relative risk for bladder cancer amongst consumers of products containing saccharin. This investigation was based on 632 bladder cancer patients and a series of matched controls. An increased risk for males consuming saccharin ($RR = 1.6$) which only just attained statistical significance ($p\ 0.02-0.03$) and a decreased risk for females ($RR = 0.6$) was reported. The numbers of saccharin consumers was small and an evaluation of this study found that the result obtained could have been due to chance, confounding factors not included in the analysis, or residual effects of those confounding factors that were considered (IARC, 1980). A more recent study of 826 bladder cancer cases and 792 randomly selected matched controls, conducted by the same authors, failed to confirm the increased risk of bladder cancer for consumers of artificial sweeteners reported earlier (Risch *et al.*, 1988).

(2) The levels of human exposure, especially in infants and children

Because of the ban on the use of saccharin in foods and beverages in Canada and the labelling requirement for foods or beverages containing saccharin in the U.S., saccharin has been eliminated from most of these products. There are no recent consumption data for saccharin in these countries, and such data would unlikely reflect saccharin consumption if this sweetener again came into general use. Nevertheless, information is available which indicates that reuse of saccharin in foods and beverages would be unlikely to pose safety concerns in the population or more specifically in infants and children.

During recent years the introduction of a number of new low calorie sweeteners has completely changed the diet food and beverage market. Approved sweetener additives in the U.S. now include aspartame, acesulfame K, sucralose and saccharin. Coincident with the availability of these new sweeteners, food and beverage technology used in sweetening these products has also advanced. The result of these changes is the concept of multisweetener use, a reduction in the concentration of any one sweetener in particular foods and beverages and a decrease in the possibility that the ADI for a given sweetener will be exceeded. The use of multiple sweeteners in foods and beverages, particularly soft drinks, is advantageous not only

to reduce daily intake levels but also because combinations of sweeteners in blends achieve a sweetness more like that of sucrose than is the case with individual sweeteners; mask after tastes; prevent delay in the onset of sweetness; and improve product stability and cost effectiveness since many low calories are synergistic in combination. There are thus not only safety reasons, but sound practical business reasons for using sweeteners in combination.

In countries where several sweeteners are available, soft drink manufacturers, the major users of low calorie sweeteners, have been quick to employ them in blends in their products. In Canada, the U.K. and other European countries, diet soft drinks are sweetened with combinations containing two or more of aspartame, acesulfame K, sucralose or saccharin. Consumption studies of low calorie sweeteners in these countries demonstrate that the individual ADI's of these additives are not being exceeded. In the U.K. a consumption survey was conducted in June 1990, at a time when acesulfame K, aspartame and saccharin were employed in beverages and as table top sweeteners. The 90th percentile consumption levels of these additives were below their respective ADI's. At that time the ADI for saccharin was 0-2.5 mg/kg bw; the corresponding value at this time is 0-5 mg/kg bw (JECFA and Great Britain).

A similar survey of low calorie sweetener consumption in Denmark was conducted during April to June 1991. At the time, the sweeteners that were approved included aspartame, acesulfame K, saccharin and cyclamate. Consumption of these sweeteners at the 90th percentile was consistently below their respective ADI's for the 1-5 and 6-9 age groups.

A survey of low calorie sweetener consumption in Germany in 1988-89 has also been reported. The results of this survey are not comparable to those in the U.K. and Denmark since cyclamate and saccharin were the only additives in general use. Aspartame was permitted only on the basis of regulatory exemption and products containing acesulfame K were not yet available. Although there were occasional children (-5 yrs. of age - 2.5%; 6-13 yrs. of age - 3.1%) whose consumption of cyclamate exceeded the ADI for cyclamate, none of the respondent children in the survey had consumption levels that exceeded the ADI for saccharin.

Given the current industry approach to the use of low calorie sweeteners and the evidence of acceptable intake levels of saccharin in countries where saccharin is in wide general use, it is highly unlikely that delisting of saccharin from the NTP report on carcinogens would result in consumption levels of saccharin in infants and children in the U.S. that would exceed the ADI.

(3) Information addressing the mechanism of urinary bladder tumor formation in male rats as it relates to other species (especially female rats and male and female mice) and to humans

It was clear from early research that saccharin tumorigenicity could not be explained on the basis of genotoxicity or covalent bonding with DNA, nor were there impurities in saccharin with these properties. There was also no evidence that saccharin was metabolized to a reactive intermediate. The key study which eventually led to an understanding of saccharin effects was

the demonstration by Hasegawa and Cohen (1986) that the saccharin salt forms differed markedly in the responses they elicited when fed to the male rat. Urothelial changes were severe and consistent with sodium and potassium salts and mild or absent with the calcium salt and acid form. This and subsequent studies focussed attention on physiological changes in the urine - high pH levels and sodium concentration, high urine volume and low osmolality, high protein concentrations (particularly in the male rat) and high urinary calcium and phosphate levels - as obligatory intermediate factors in the pathogenesis of urothelial change. Eliminating excess sodium from the diet, i.e. by feeding acid saccharin; or feeding sodium saccharin in a diet which results in an acidic urine (i.e. the AIN-76A diet or a diet containing ammonium chloride) abolished the urothelial effects and tumor promoting effects of saccharin. When the critical urine physiological changes are present in the male rat, a flocculent white precipitate develops in the urine and it is this precipitate which is believed to be responsible for the urothelial changes that are observed and, ultimately, tumor development.

The bladder tumors that occur in rats fed high levels of sodium saccharin develop primarily in the male rat. Some tumors have been observed in females but clearly the female rat is much less sensitive to tumor development. Differences in the physiological urinary changes and urinary precipitate in male and female rats in response to saccharin explain this gender difference in response (Cohen, 1997). Similarly, differences in urinary physiological change in response to feeding sodium saccharin provide an explanation for the lack of a tumorigenic response in mice, in some strains of rats such as the NBR rat (Uwagawa, 1992), and in the monkey. The specificity of the critical urinary changes for the male rat and the differences between rat and human urine with respect to these factors mitigate strongly against sodium saccharin being a bladder tumorigen for humans.

In a consideration of the effects of sodium saccharin on the bladder of the male rat, one must also include the findings from studies of other related sodium salts. Studies in Japan have shown that sodium ascorbate causes urinary physiological changes, urothelial damage and tumor promotion in the male rat over a dose range comparable to that of sodium saccharin. In a study that has been accepted for publication, Cohen *et al.* have shown that feeding high levels of sodium ascorbate to the male rat also causes bladder tumors. These findings demonstrate that sodium ascorbate and sodium saccharin share a common tumorigenic mechanism. Although studied to a more limited extent, it is known that a wide variety of sodium salts including those of phosphate, citrate, aspartate, succinate, erythorbate and glutamate produce urinary and urothelial changes comparable to those associated with sodium ascorbate and sodium saccharin when fed at high levels to the male rat. It is likely that these sodium salts would also cause bladder tumors if fed at high levels to the male rat in long-term studies. The bladder changes produced by sodium salts are non-specific and the result of the inability of the rat to physiologically accommodate to the massive overloads of the substances involved.

(4) The adequacy of data for tumor formation in laboratory animals at target sites other than the urinary bladder.

Long-term studies of saccharin have been reported in mice, rats, hamsters and monkeys and studies have been done to determine whether statistically significant tumor incidences were present in tissues other than the bladder.

Hamster

A long-term study (80 weeks) of sodium saccharin at levels up to 1.25% in the drinking water was reported by Althoff *et al.*. There were no statistically significant differences in tumor incidences in any tissues between treated animals and controls.

Monkey

Two long-term studies of sodium saccharin in monkeys have been reported. In the first of these studies (Coulston *et al.*, 1978) sodium saccharin was administered by gavage to Rhesus monkeys six days a week for 19 months at doses up to 500 mg/kg bw. Histopathological examination did not reveal tumors in treated or control monkeys. In the second study (Thorgeisson *et al.*, 1994; Takayama *et al.*, 1998) sodium saccharin was fed to 20 monkeys of this species at a dietary level of 25 mg/kg bw 5 days a week for 24 years. Sixteen monkeys served as controls. At necropsy, tumors were observed in three of the treated monkeys: in the first a thyroid lymphoma, in the second a leiomyoma of the uterus and in the third a cystadenoma of the ovary and a leiomyoma of the stomach; there were no bladder tumors. All of these tumor types are observed in normal control and breeding animals in the same colony.

Rat

One is hard pressed to recall any substance that has been as exhaustively studied in long-term bioassays as saccharin. This sweetener has been the subject of 13 single-generation studies in the rat and four two-generation studies. The bioassays have been reported during a period from 1951 to 1986 and have been the subject of numerous reviews. Insofar as mouse and rat studies conducted prior to 1974 are concerned, a committee of the National Academy of Science concluded that "In none of the studies were there tumors of tissues, with the possible exception of the urinary bladder, that showed an association with saccharin intake" (Safety of saccharin and sodium saccharin in the human diet, National Academy of Sciences, 1974). Similarly, a review by the Office of Technology Assessment entitled Cancer Testing Technology and Saccharin (1977) concluded only that with respect to studies conducted in mice, rats, hamsters and monkeys "the two-generation experiments (rats) showed that saccharin caused an increase in bladder cancer and especially among males". A further NRC/NAS committee that assessed saccharin in 1978 also concluded that "in single generation studies (11 studies including mice, rats, hamsters and monkeys) saccharin did not induce cancer in any organ". Saccharin was found to be a bladder carcinogen in male rats in two-generation studies (Saccharin: technical

assessment of risks and benefits, Report No.1, Committee for a Study on Saccharin and Food Safety Policy, NRC/NAS, 1978).

The reviews of saccharin cited above did not include the more recent bioassays in the rat reported by Chowanec and Hicks (1979), Arnold *et al.* (1980), Schoenig *et al.* (1985) and West *et al.* (1986). In none of these studies was there an increased tumor incidence in any organ of saccharin-treated animals other than in the bladder. The many single and two-generation lifetime studies of saccharin in the rat demonstrate conclusively that the tumorigenic effects of this sweetener are directed solely at the bladder.

Mouse

As indicated above, long-term studies of saccharin conducted prior to the mid-seventies in the mouse have been reviewed by several NRC/NAS committees that have concluded that saccharin does not cause cancer in this species. Since that time, bioassays of saccharin in the mouse have been reported by Kroes *et al.* (1977), Prasad and Rai (1986) and Frederick *et al.* (1989). Kroes *et al.* (1977) did not observe any carcinogenic effect of saccharin in any organ in a multigeneration study in mice in which saccharin was fed at dietary levels of 0.2 and 0.5%. Frederick *et al.* (1989) fed saccharin to mice in the diet at levels of 0, 0.1, 0.5, 1.0 and 5.0% for 132 weeks. There were no significant differences in tumor incidences between control and treated mice in any organ.

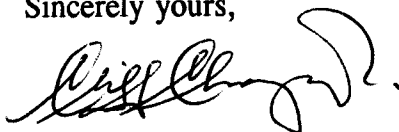
Of the six long-term bioassays of saccharin in the mouse that have been reported, the only one the results of which suggest a carcinogenic effect of saccharin is that of Prasad and Rai (1986). These investigators fed saccharin by gavage to mice for one year; the duration of the other studies ranged from 18 to 30 months. Prasad and Rai reported that 5/10 male and 2/10 female mice fed 1.5 g/kg bw saccharin for one year developed protruberant neck tumors that on histopathological examination were papillary adenocarcinomas. No tumors were observed in mice dosed with 0.5 or 1.0 g/kg bw of saccharin. In a second report of this study the authors reported that the mice fed 1.0 and 1.5 g/kg bw of saccharin had a severe normochromic hypocytic anemia and a marked increase in polymorphonuclear neutrophils.

It is doubtful that the Prasad and Rai studies can be taken at face value. It is inconceivable that a thyroid tumorigenic effect and severe anemia would have been missed in all the other studies of saccharin in the mouse, several of which were twice the duration of the Prasad and Rai study and employed higher saccharin dose levels. Furthermore, the study is flawed by virtue of the test material used. Prasad and Rai (1987) described the saccharin as a "commercial preparation" obtained from a pharmaceutical concern (Boots Co., Bombay, India). Contrary to the minutes of the NTP Board Subcommittee meeting, the saccharin salt was not identified, and the authors stated that "no attempt was made to purify" this material. Although one cannot be certain, the information provided suggests that a tabletop sweetener dissolved in water was used to dose the mice. The control mice were not dosed with the ingredients of the tabletop sweetener other than the saccharin. On this basis alone one must discount the validity of this study.

The weight of evidence demonstrates clearly that saccharin is not a carcinogen in any organ in the mouse. There is no reliable evidence to suggest that saccharin is a carcinogen in any of the four species tested in any organ other than the bladder tumors reported in the rat.

None of the many scientific reviews of saccharin carcinogenicity in the rat published during recent years has concluded that saccharin consumption poses a cancer risk for man. It is important to the integrity and image of the National Toxicology Program that decisions taken with respect to saccharin should reflect the vast body of evidence that attests to the safety of this sweetener and also the widely held views of the scientific community.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Cliff Chappel", with a stylized flourish at the end.

Clifford I. Chappel, D.V.M., Ph.D.